A NOTE ON THE TOXICITY OF ACIDS FOR MOSQUITO LARVÆ.

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Interest in the resistance of mosquito larvæ to various physical and chemical reagents has been largely, if not solely, directed toward the discovery of practical methods for the eradication of the mosquito. A large amount of literature dealing with this phase of the subject is now available. The effects of various solutions of salts on mosquito larvae have also been rather extensively investigated and it has been found that the animals are able to withstand rather high concentrations of pure salt solutions (Mac Fie (1), Chidester (2), Sen (3), Sharma (4), etc.). It has recently been pointed out by MacGregor (5) that mosquito larvæ are able to live and develop in extremely high concentrations of acid, *e.g.*, acid of $P_H = 4.4$. In view of these last mentioned observations, it was thought advisable to test the toxicity of a series of acids of different concentrations for mosquito larvæ and the present paper embodies results obtained from such experiments.

The larvæ used in all experiments, *Culex pipiens*, were obtained in large numbers from small, stagnant pools usually found in uncovered containers. The entire culture as found was brought into the laboratory and tests carried out at the same time and with the same group of animals, all of which had presumably been under identical conditions. Both young and old larvæ were used and differences due to age noted. The animals were removed from the cultures by means of a wide-mouth pipette and transferred to a syracuse watch glass with as little of the culture medium as possible. In this way a large number of larvæ could easily be obtained for use in each experiment. Fifty to sixty animals were used in testing the effect of any concentration of reagents. The chemicals used (10 c.c.) were put into covered syracuse watch glasses; the larvæ were quickly injected into the solution and then observed until dead under a binocular micro-

scope. The fatal exposure was taken at that time when approximately one half of the larvæ were killed, *i.e.*, when movements of the heart and alimentary canal ceased. Many cultures were used in experiments and slight variations in their resistance were shown but in the following only average results will be given. It is, however, of some interest to note that in practically all the cultures from which larvæ were obtained the hydrogen ion concentration showed them to be neutral or slightly alkaline in reaction (P_H = 7.0–7.4). The chemicals used were, hydrochloric, acetic, oxalic, butyric, salicyclic and carbonic acids and mercuric chloride.

Fatal Exposures in Minutes to Different Strengths of Acids (Temp. 25° C.).

Normality of Acid.	HCl.	Oxalic.	Salicyclic.	Butyric.	Acetic.
0.5	9.5	.....	.....	.....	.....
0.2	42.0	.....	.....	.....	.....
0.1	74.0	39.0	.....	72.0	191.0
0.01	293.0	52.0	48.0	1440.0	1440.0+
0.001	1440.0+	1440.0	1200.0	1440.0+	1440.0++
0.0001	.....	1440.0+	1440.0+	.....	.....

From the above table showing the length of life in minutes of larvæ in various strengths of acids it is evident that the animals are able to withstand abnormally high concentrations of acids for rather long periods of time. This remarkable resistance of mosquito larvæ is more strikingly shown when compared with that found for other forms—*e.g.*, Honda (6) found that the free-living nematode *Rhabditis elegans* withstood 0.01 normal HCl for 60 minutes, *Daphnia* for 23 minutes, tadpoles for 12 minutes and paramecium for 1 minute. (Personal communication to be published in *Journal of Experimental Zoölogy*.) MacArthur (7) found that Planarians are killed in a very short time by exposure to HCl of P_H 2–4.5. It has also been found by the author (8) that cysts of *Colpoda* withstand 0.001 N HCl for a strikingly long time.

That the hydrogen ion concentration is not necessarily the only factor in the toxicity of acids for larvæ is shown by comparing the effects of a saturated solution of CO_2 in H_2O of a P_H of approximately 3.7 with a solution of HCl of the same P_H value.

Larvæ in the CO_2 solution become motionless almost at once and the movements of heart and alimentary canal also quickly cease, while in HCl of the same P_H value they are apparently unaffected for over 24 hours. The more rapid penetration rate and mode of action of CO_2 as pointed out by Jacobs (9), doubtless account for the differences observed in the effect of the two reagents.

It is also of much interest to know in what manner the acids kill the animals, whether they enter the chitinous covering or enter by the mouth or anus through the alimentary canal. By using pupae, which are known not to eat nor to have external openings as in the larvæ, it is found that the acids do not kill them for many hours, considerably in excess of the lethal exposure for larvæ. From this fact it seems reasonable to assume that the larvæ are killed by the entrance of the reagent orally rather than cutaneously. The present discussion, however, deals primarily with the resistance of the animals to the reagents rather than with their mode of killing. Younger and smaller larvæ, and these doubtless have thinner chitin, are killed somewhat more quickly than older individuals.

The general order of toxicity of the acids used for the larvæ is, salicyclic > oxalic > HCl > butyric > acetic. This series is strikingly similar to that found by Haas (10) for plants, by Collett (11) for protozoa and by Bodine (8) for cysts of *Colpoda*.

Fatal Exposures in Minutes to Different Percentages of $HgCl_2$ (Temp. 25° C.).

Per Cent. $HgCl_2$.	Time in Minutes.
0.05	305.0
0.10	155.0
0.50	48.3
1.00	26.5
2.00	20.0

Mercuric chloride in various concentrations was used and here again the resistance of the larvæ is of considerable interest. The above table shows the effect of different percentages of this salt. Honda (6) found that the free-living Nematode *Rhabditis elegans* withstood 0.05 per cent. $HgCl_2$ for 60 minutes; *Daphnia*, 25 minutes and tadpoles, 5 minutes. Mosquito larvæ are about 5 times as resistant to $HgCl_2$ as the most resistant form used by this author. Sen (3), with other species of mosquito larvæ (*Ste-*

gomyia albopicta) found that the animals were killed at once in a 0.1 per cent. $HgCl_2$ solution. This difference in length of time of fatal exposure to this concentration from the present result is doubtless due to the differences in resistance of the two species as well as to the end point taken in the experiments. Sen evidently used cessation of body movement while in the present investigation cessation of movements of heart and alimentary canal were taken as the end point.

Summary.

1. Mosquito larvæ (*Culex pipiens*) were found to be extremely resistant to rather high concentrations of various acids.

2. The order of toxicity of the acids used is, salicylic > oxalic > HgCl > butyric > acetic.

3. The chemicals seem to penetrate the animal orally and not cutaneously.

4. Animals withstand rather high concentration of $HgCl_2$, considerably in excess of that found for other organisms cited.

Literature Cited.

1. **Mac Fie.**
'21 Ann. Trop. Med. & Parasit., Vol. XV., No. 4, p. 377.
2. **Chidester**
'17 N. J. Agric. Expt. Sta., Bull. 299.
3. **Sen.**
'21 Rept. Proc. 4th Ent. Meeting, Pusa, Calcutta, p. 184.
4. **Sharma.**
'21 Rept. Proc. 4th Ent. Meeting, Pusa, Calcutta, p. 199.
5. **MacGregor.**
'21 Parasitology, Vol. 13, No. 4, p. 348.
6. **Honda.**
Journal Exp. Zoölogy (in press).
7. **MacArthur.**
'20 Am. Jour. Physiology, Vol. LIV., p. 138.
8. **Bodine.**
'23 Journal Exp. Zoölogy (in press).
9. **Jacobs.**
'20 Am. Jour. Physiology, Vol. LI, No. 2. p. 321
10. **Haas.**
'16 Journal Bio. Chem., Vol. 27, p. 225.
11. **Collett.**
'19 Journal Exp. Zoölogy, Vol. 29, No. 3, p. 443.